The Pathophysiological Effects of Moraxella bovis Toxins on Cattle, Mice and Guinea Pigs

G. W. Pugh, Jr., D. E. Hughes and V. D. Schulz*

ABSTRACT

In three experiments, cattle, mice and guinea pigs were inoculated with viable cultures of Moraxella bovis or fractions of this organism. Fractions were obtained by disruption of cells with a fractionator at 20,000 pounds per square inch, and separating the cell wall and cell sap fractions by differential centrifugation. Cell sap fractions were further separated by ultracentrifugation, heating and precipitation with $(NH_4)_2$ SO_4. Different fractions induced different pathophysiological manifestations. The cell wall fractions caused localized lesions (necrosis) at the site of injection, and emphysema and congestion of the lungs. Cell sap fractions induced a "shock syndrome," as well as hemorrhage and inflammation of the intestines, hemorrhage and congestion of lymph nodes, liver, adrenal and spleen. Cell sap also induced conjunctivitis in mice and guinea pigs, and periocular edema, myosis, ocular pruritus and lacrimation in cattle.

The authors suggest that M. bovis probably produces endotoxins and exotoxins as well as possibly a specific oculopathic substance, but more definitive work is needed to confirm this. They caution that consideration of these toxins should be made in any application of M. bovis for vaccines or other immunological studies.

*National Animal Disease Laboratory, North Central Region, Agricultural Research Service, U.S. Department of Agriculture, Ames, Iowa 50010.

Submitted February 21, 1972.

RÉSUMÉ

Au cours de trois expériences, on inocula des bovins, des souris et des coyabes avec des cultures vivantes de Moraxella bovis ou avec des extraits de ce microbe. On obtint les extraits en brisant les cellules à l'aide d'un appareil à fractionner, sous une pression de 20,000 livres au pouce carré, et en séparant la membrane cellulaire de la sève, au moyen de la centrifugation différentielle. On sépara ensuite les constituants de la sève cellulaire en ayant recours à l'ultracentrifugation, à la chaleur et à la précipitation à l'aide de $(NH_4)_2$ SO_4. Ces différentes fractions provoquèrent autant de manifestations pathophysiologiques. Les constituants de la membrane cellulaire causèrent des lésions locales (nécrose) à l'endroit de l'injection, ainsi que de l'emphysème et de la congestion pulmonaires. Les constituants de la sève provoquèrent un "syndrome de choc", ainsi que des hémorragies et une inflammation intestinales, de la congestion et des hémorragies dans les ganglions lymphatiques, le foie, les surrénales et la rate. Le sève cellulaire cause aussi de la conjonctivite chez les souris et les cobayes, ainsi que de l'oedème périoculaire, de la myose, du prurit oculaire et du larmoiement, chez les bovins.

Les auteurs pensent que M. bovis élaborerait des endotoxines et des exotoxines, ainsi qu'une substance oculopathique spécifique; à leur avis, la confirmation de cette hypothèse nécessitera cependant une étude plus poussée. Ils signalent aussi qu'il faudrait tenir compte de ces toxines lorsqu'on utilise M. bovis pour produire des vaccins ou pour effectuer d'autres études immunologiques.

INTRODUCTION

Although it is generally accepted that *Moraxella bovis* is the etiological agent of bovine infectious keratoconjunctivitis (BIK), very little work has been done on the toxic effects of this organism and its products. Thus, a dearth of information exists in spite of the fact that some investigators have suggested that toxins of *M. bovis* may account for the severe ocular lesions in BIK (6, 12) or may be pertinent in the development of vaccines (7). Some investigators have also shown that viable cells of *M. bovis* injected intramuscularly, intradermally or into the scrotum cause severe necrosis (5, 6, 8, 12, 14, 15) and, in some cases, systemic effects. Viable *M. bovis* cultures injected into mice, rabbits and chicken embryos produced changes which were considered consistent with the presence of one or more toxins (6).

In light of the fact that a recent report (7) has indicated that immunization against BIK is feasible, but the use of viable cultures of *M. bovis* may be hazardous because of toxic-like reaction, there was a need to understand the toxic effects of *M. bovis* and its fractions. This report represents the results of a study undertaken to facilitate the development of a safe vaccine against BIK.

MATERIALS AND METHODS

Experimental Design

The study was conducted in five parts (experiments Ia, Ib, Ic, IIa, and IIb). Experiments Ia-Ic were designed to determine the toxic effects of whole cells, cell sap and cell wall preparations of *M. bovis* in cattle. Experiments IIa and IIb were designed to determine the toxic effects of *M. bovis* and its fractions in mice and guinea pigs, respectively.

Experiment Ia

Whole cell preparation — Strain EPP-63(300) *M. bovis* was used as the test organism. This strain was isolated from a steer naturally affected with BIK, and on the basis of results of other studies was proven to induce keratoconjunctivitis in cattle (7, 12, 14) and mice (12). The organism was also shown to be immunogenic although it had toxic properties (7). Cultures of the organism were stored at -60°C in vials of litmus milk.

Viable whole cells for injection into cattle and preparation of fractions were started from the frozen cultures. A culture was thawed and streaked on 5% bovine blood agar (BBA); the agar plate cultures were incubated at 37°C for 24 hours and then at 25°C for 24 hours. Isolated colonies were streaked on additional plates that were incubated at 37°C for 24 hours. Bacterial growth on these plates was scraped off and either stored at -60°C for later fractionation procedures or washed twice in physiological saline solution (PSS) and resuspended in PSS to give a suspension approximating the density of a MacFarland nephelometer number 3. Five ml of the latter suspension was injected into the jugular vein of each of two cattle.

Cattle — Two Holstein-Fries; an bulls in good health, weighing approximately 700 lbs each were used. They had recovered from experimental BIK. The sera of both had precipitating (12) antibodies against *M. bovis.*

Experiment Ib

Exposure Material (Cell Fractions) — Soluble and insoluble crude fractions of *M. bovis* for giving injections to animals in experiments Ib, Ic, IIa and IIb were prepared from strain EPP-63(300) in the following manner. A paste of cells consisting of 90 gm of cells harvested from 24-hour BBA cultures was suspended in 123 ml of cold distilled water. The cells were further diluted with water to facilitate the passing of the suspension into the fractionator.[1] The cells were disrupted in the fractionator, and the effluent was separated differentially in low speed[2] and high speed[3] centrifuges. The summarized procedure is given in Fig. 1. All procedures were carried out in the cold.

---

[1]Sorvall Ribi Cell Fractionator — Model RF-1, Ivan Sorvall, Inc. Norwalk, Connecticut, U.S.A.

[2]International portable refrigerated centrifuge, Model PR-2, International Equipment Company, Boston, Mass.

[3]Beckman Model L preparative ultracentrifuge, Beckman Instrument Inc., Palo Alto, California.

Cattle — Three healthy Holstein-Friesian calves each weighing approximately 700 lbs were used. They were culturally negative for *M. bovis*, and their sera were free of precipitating antibodies to this organism.

Exposure — Each of two calves was given 1.0 ml of fraction P-3 intravenously on day one, and on day 14 one was given an additional 1.0 ml and the other 2.0 ml. The third calf was given 1.0 ml of fraction CW-2 which had been diluted to give a density equal that of a MacFarland nephelometer number 4, intramuscularly. Fourteen days later an additional 2.0 ml was given.

EXPERIMENT Ic

Exposure materials — The exposure materials used were P-1 and CW-2. Portions of P-1 and CW-2 were heated to 85°C for two hours and designated fractions P-1H and CW-2H, respectively. Cell wall preparations had a density which equalled that of a MacFarland nephelometer number 4.

Cattle — Four yearling Holstein-Friesian cattle were used. They had been used in another experiment where they had been vaccinated against BIK using viable cultures of *M. bovis*. They had precipitating serum antibodies for *M. bovis;* all had become infected with *M. bovis* after challenge, but only one had developed BIK. At the time of exposure, however, all were normal and culturally negative for *M. bovis*. They weighed approximately 700 lbs each when they were used in this experiment.

Exposure — Two calves were given cell sap material by jugular venipuncture. Calf 6400 was given 5.0 ml of fraction P-1 initially, and 90 minutes later an additional 3.6 ml were given. Calf 6424 was given 5.0 ml of fraction P-1H at time zero and 3.5 ml 90 minutes later.

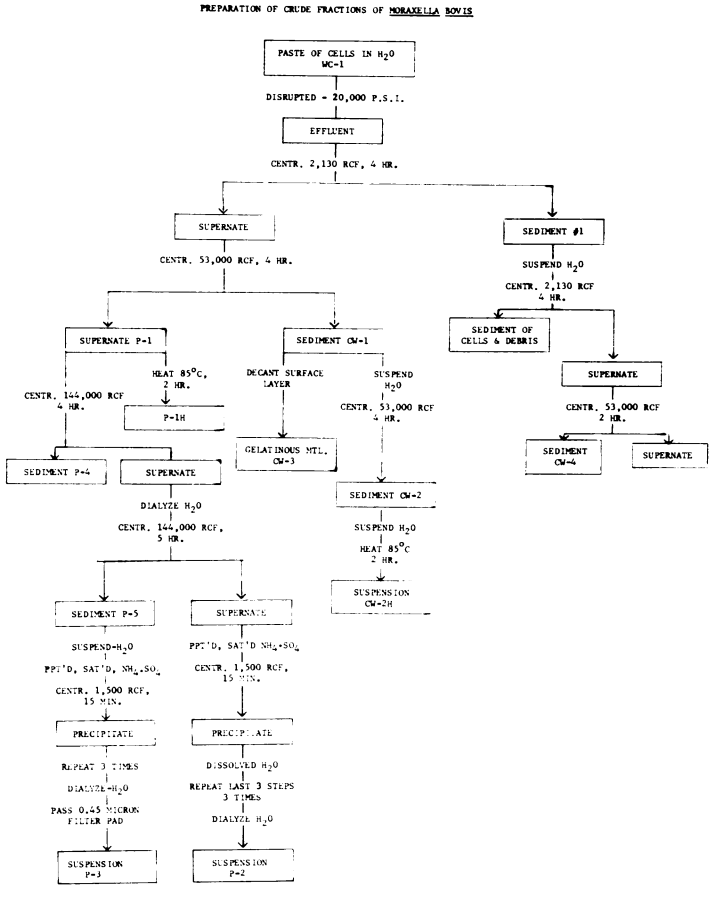

Fig. 1. Flow chart of the procedure followed in the preparation of fractions of Moraxella bovis for pathophysiological studies. Abbreviations used are (PSI = pounds per square inch, Centr = Centrifuged, RCF = relative centrifugal force, PPT = precipitate, and $NH_4 \cdot SO_4$ = $(NH_4)_2SO_4$).

Two other calves were given cell wall preparations by jugular venipuncture. Calf 6314 was given 5.0 ml of fraction CW-2 initially and 3.5 ml after 90 minutes. Calf 6325 was given 3.3 ml of fraction CW-2H initially and 3.5 ml 90 minutes later.

EXPERIMENT IIa

Exposure material — Whole cells and fractions of *M. bovis* for exposure of mice were as described in Experiments Ia and Ib, respectively. The whole cells were viable and were diluted in trypticase soy broth[4] to give a plate count (13) of 2.5 x 10^5 organisms per dose (0.2 ml). The fractions were culturally negative for microorganisms. Fractions and their sources are listed in Table I.

Mice — One hundred and thirty-two female mice each weighing from 16 to 24 gm were used. All were healthy and had not been exposed to *M. bovis* or fractions of this organism previously.

Exposure — The number of mice exposed and the exposure material are listed in Table I. Each mouse was given 0.2 ml of a specific exposure material intravenously via the lateral tail vein.

EXPERIMENT IIb

Exposure materials — Fractions CW-2 and P-5 as described in Experiment IIa were used.

[4]Trypticase Soy Broth, Baltimore Biological Laboratories, Baltimore, Md.

Animals — Twenty-four healthy guinea pigs each weighing approximately 400 gm were used. They had never been exposed to *M. bovis* or its products previously. The guinea pigs were placed in three groups of eight guinea pigs each.

Exposure — Guinea pigs in Group 1 were exposed to cell sap P-5, those in Group 2 to cell wall fraction CW-2 and those in Group 3 to an equal-portion mixture of fraction CW-2 and cell sap P-5. Each guinea pig was exposed to 0.5 ml of the material that its group was to receive. A dose of 0.25 ml was injected intramuscularly into each hip of every animal.

RESULTS

EXPERIMENT Ia

Immediately after washed *M. bovis* cells were injected into cattle, the following manifestations were observed: rapid (80/min) shallow breathing mydriasis, increased serous lacrimination, frothing from the mouth, hacking cough and micturation. Within 20-30 min the respiratory distress had increased; there was blood flecked nasal and oral froth, and the animals began to bloat. At this time, epinephrine was administered, but the signs became progres-

TABLE I. Exposure of Mice to Whole Cells and Various Fractions of Moraxella bovis

Source of Exposure Material	Code Designation	Optical Density	Number of mice Exposed
Viable *M. bovis* culture	WC-1	MN[a] No. 4	10
Cell walls — unwashed	CW-1	MN No. 4	10
Cell walls — washed	CW-2	MN No. 4	10
Cell walls — washed and heated[b]	CW-2H	MN No. 4	10
Gelatinous cell wall material	CW-3	MN No. 3	10
Cell walls — low speed (2130 RCF) washings of larger particles	CW-4	MN No. 4	12
Cell sap — unheated	P—1	PH[c,d] 80	10
Cell sap — heated	P-1H	PH 80	10
$(NH_4)_2SO_4$ Precipitated cell sap	P-2	PH 60	10
$(NH_4)_2SO_4$ Precipitated and filtered cell sap	P-3	PH 40	10
Ultracentrifugation sediment of cell sap	P-4	PH 85	10
Ultracentrifugation sediment of dialyzed cell sap	P-5	PH 30	10
Trypticase soy broth	Control		10

[a]MN — MacFarland Nephelometer
[b]Fractions CW-2H and P-1H were heated at 85°C for two hours
[c]PH — photo-nephelometer model 7, Coleman Instruments, Inc., Maywood, Illinois
[d]The nephelometric procedure and standard use were as described (2)

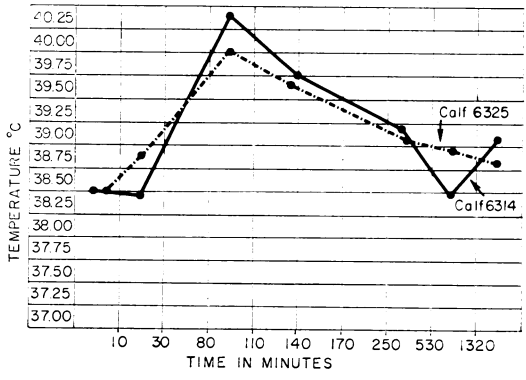

Fig. 2. Comparison of the temperature responses of two calves given cell wall fractions of Moraxella bovis. Calf 6314 was given unheated cell wall fraction CW-2. Calf 6325 was given heat-treated fraction CW-2H. The six-day average temperatures before giving injections to calves 6314 and 6325 were 38.5° and 38.6°, respectively.

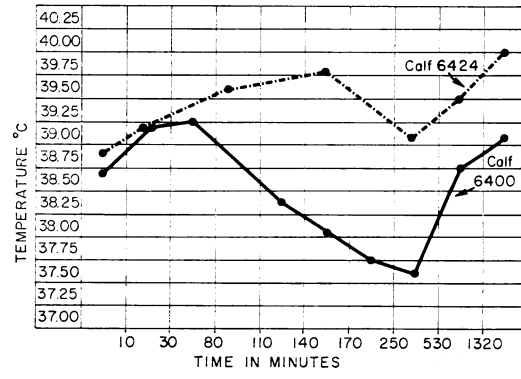

Fig. 3. Comparison of the temperature responses of two calves given cell sap of Moraxella bovis. Calf 6400 was given unheated cell sap P-1 and calf 6424 was given heat-treated cell sap P-1H. The six day average temperatures before giving injecion to cattle 6400 and 6424 were 38.4° and 38.9°, respectively.

sively more severe and both cattle became recumbent. Subsequent intramuscular injections of epinephrine and antihistamine did not ameliorate the condition. Both cattle became moribund and died within ten hours. Postmortem lesions were limited to the respiratory tract. These lesions were similar to those described later under Experiment Ic.

Experiment Ib

Immediately after injection of cell sap P-3 into calves 6418 and 6444, they had respiratory distress. Their respiratory rates were 72 and 90 per min, respectively. They had intermittent hacking coughs and were highly agitated. The signs gradually subsided so that, after 24 hours, calf 6418 was back to normal, but calf 6444 was lethargic, scouring and anorectic. Calf 6409 which was given fraction CW-2 did not develop clinical signs.

Experiment Ic

The results are summarized in Figs. 2 and 3 and Tables II and III. However, it should be noted that the cattle given the cell wall fractions had noticeable, acute respiratory signs (Table II), and the most prominent lesions on postmortem examination were limited to the respiratory tract. The lungs were emphysematous, and there was frothy material in the trachea and bronchi. The lesions and signs (Fig. 2 and Table II) of the animal given the unheated cell wall fractions were more severe than the one getting the heat-treated fraction. In the former, there were petechial hemorrhages on visceral lymph nodes, cecum, and intestine and kidney.

The signs in the animal given unheated protoplasm fraction (P-1) were respiratory, digestive, ocular, as well as nervous (Table III). On postmortem examination of the calf, which received unheat-treated fraction P-1, the respiratory tract was practically normal but severe digestive tract lesions were evident. There were ecchymotic hemorrhages in the proximal third of the small intestine. There also were petechial hemorrhages in the cecum, ilium, kidney, visceral lymph nodes, adrenal glands and the urinary bladder. The liver and spleen were swollen and friable. The postmortem lesions of the calf receiving heat-treated fraction P-1 were less severe. The hemorrhage was found in the same organs as seen in the other calf, but it was only of the petechial type. The respiratory system was normal.

Experiment IIa

The results obtained with mice given *M. bovis,* whole cells and fractions are summarized in Table IV. Mice given whole cell cultures became lethargic, developed diarrhea and became moribund and died within 72 hours. The mice given cell wall fractions developed severe inflammation at the site of injection. The inflamed areas sloughed in some cases but the tail did not fall off. These mice did not consume appreciable amounts of feed and water until the third day after injection.

TABLE II. Sequential Clinical Manifestations of Calves Given Cell Wall Fractions of Moraxella bovis

Time after Exposure	Signs Manifested	
	Calf 6314[a]	Calf 6325[b]
0	Resp. rate (70/min), salivation, staggering	Deep intermittent cough, resp rate (30/min), eyes normal
10 min	Dyspnea, mild bloat, eyes normal	Resp rate (70/min), expiratory grunt, frothing, defecation
30 min	No change	No change
80 min	No change	No change
110 min	Micturation, straining, trismus bloat	Signs more severe, excited, moderate bloat
140 min	Increased resp distress, otherwise no change	Severe bloat, otherwise normal, eyes normal
170 min	No change	No change
250 min	Severe dyspnea, expiratory grunt, eyes normal	Dyspnea reduced, lethargy, frothing from mouth
530 min	Blood-flecked oral froth, otherwise no change	Improved, eyes normal
1320 min	Lethargic, otherwise normal, killed	Lethargic, otherwise normal, killed

[a]Given unheated cell wall fraction CW-2
[b]Given heated cell wall fraction CW-2H

TABLE III. Sequential Clinical Signs Exhibited by Calves Given Cell Sap Materials of Moraxella bovis

Time after Exposure	Signs Manifested	
	Calf 6400[a]	Calf 6424[b]
0	Panting, defecation, ocular pruritus	Similar to 6400 but less severe
10 min	Excess lacrimation, severe periorbital edema, micturation	Defecation, hyperpnea, cough, lethargy, lacrimation
30 min	Bloat, cold scrotum, myosis, periorbital edema	Hyperpnea (80/min), bloat
80 min	Expiratory grunt, trembling, recumbency	Dyspnea, recumbency
110 min	No change	No change
140 min	No change	No change
170 min	No change	No change
250 min	Moribund, copious fetid diarrhea	No change
530 min	No change	Respiration normal, fetid diarrhea
1320 min	Copious chocolate colored feces, killed	Almost normal, killed

[a]Given unheated cell sap P-1
[b]Given heated cell sap P-1H

TABLE IV. Clinical Signs Exhibited by Mice Given Whole Cells or Fractions of Moraxella bovis

No. of Mice	Fraction	Death[a]	Anaphylactoid-like Reaction	Conjunctivitis	Ruffled Hair Coat	Diarrhea
10	CW-1[b]	10	5	10	10	10
10	CW-1	0	0	10[c]	10	3
10	CW-2	0	0	0	0	0
10	CW-2H	0	0	0	0	0
10	CW-3	5	4	5	10	10
12	CW-4	12	5	12	12	5
10	P-1	9	0	9	9	10
10	P-1H	0	0	10	10	10
10	P-2	8	0	10	10	10
10	P-3	3	0	10	10	10
10	P-4	0	0	10	10	10
10	P-5	5	0	10	10	10
10	Control	0	0	0	0	0

[a]Death occurred from 24 to 48 hours after injection of fractions
[b]There was severe inflammation at site of injection in the mice given cell wall fractions
[c]Very mild conjunctivitis

Some mice in the three groups (Table IV) given cell wall fractions CW-1, CW-3 and CW-4 developed severe immediate anaphylactoid-like reactions after injection. A typical reaction was as follows: the mouse became hyperactive as evidenced by jumping about, scratching and rubbing its nose, holding its tail erect and stretching the body. This was followed immediately by a complete collapse of the body, and the mouse became comatose, with blanched skin and mucous membranes; breathing apparently ceased, and the eyes appeared glassy and lifeless. After ten to 30 minutes the mouse began slow intermittent deep respiratory motions, which gradually returned to normal in depth and rate. All animals gradually returned to normal within an hour with no subsequent abnormal signs.

All of the mice in the different groups exposed to cell sap, except the group exposed to heat-treated cell sap (P-1H), developed similar signs. Signs were not seen immediately after injection of the cell sap fractions, but after ten to 20 hours the mice receiving unheated cell sap developed a mild conjunctivitis with serous lacrimation. The conjunctivitis became progressively more severe and developed into a severe hemorrhagic conjunctivitis without corneal involvement. Other prominent signs were extreme lethargy, ruffled hair coat and profuse diarrhea which became hemorrhagic in many cases. Within 48 hours the mice usually were moribund, and many died within 96 hours; those not dying gradually improved and were back to normal within a week. Those mice receiving heated cell sap had mild signs such as decreased feed and water consumption and a mild conjunctivitis. There were no lesions at the site of injection in any of the mice injected with cell sap.

Experiment IIb

The immediate effect of cell sap P-5 on the guinea pigs in Group 1 was extreme lethargy with lacrimation and conjunctivitis, and two of the guinea pigs were comatose within 30 minutes after being inoculated. There was acute death (similar to shock syndrome) within five hours in these two guinea pigs. Upon necropsy, the lesions were those of generalized hyperemia and hemorrhage. The adrenal glands were noticeably hemorrhagic. The surviving guinea pigs were almost back to normal 48 hours after injection. When they were killed more than two weeks after receiving the injection, there were small abscesses in one leg of each of three of the six guinea pigs.

There were no generalized signs in guinea pigs given cell wall CW-2, but there was extreme tenderness and lameness in the hind legs; the tenderness was especially noticeable at the site of injection. No deaths occurred in this group but they did not consume appreciable quantities of feed and water until the third day after injection. When killed more than two weeks after injection, eight of 16 legs (six guinea pigs) had muscle lesions at the site of injection.

The guinea pigs which were given the combined cell wall and cell sap preparations had signs and lesions characteristic of both of the other groups but none died. When the guinea pigs in this group were killed more than two weeks after injection, 12 of 16 legs (eight guinea pigs) had muscle lesions at the injection site.

DISCUSSION

The results of experiment Ia indicate that viable cells of *M. bovis* cause pathophysiological changes in cattle when given intravenously and in large numbers. These findings support those of others (7, 8, 14, 15) who indicated that *M. bovis* may produce either local (8, 14, 15) or generalized (7, 12) toxin-like effects in cattle. Similar results have been reported for laboratory animals (5, 6, 12).

The results of experiments Ib and Ic indicate that cell walls as well as cell sap fractions of *M. bovis* are toxic, but the toxicity is diminished by heat. The results also suggest that there are at least two toxins associated with *M. bovis*, or that the toxin of *M. bovis* has more than one pathophysiological action. This contention is supported by the work of others (6) who have suggested that *M. bovis* produces more than one toxin. A preliminary analysis[5] done in conjunction with this study revealed that the cell sap preparation used in our experiments did not contain heptose, a nearly universally occurring component of bac-

[5]Rebers, P. A., and G. W. Pugh, Jr., National Animal Disease Laboratory, North Central Region, Agricultural Research Service, U.S. Department of Agriculture, Ames, Iowa 50010 (Unpublished data, 1971).

terial endotoxin (3, 9). This finding suggests that the pathophysiological effects caused by the cell sap, (i.e., lowered body temperature, ocular edema, local edema, severe enteritis and lesions in parenchymatous tissue) are due to an exotoxin. The presence of an exotoxin is inferred by the heat ability of cell sap toxic effects and the protein nature of fractions P-2 and P-3.[5,6] The production of an exotoxin is further substantiated by the results of another study (12) where cultural filtrate of *M. bovis* produced lethal effects in rabbits and embryonating chicken eggs. However, it should be pointed out that some of the exotoxin effects may be manifested in conditions due to endotoxins (1, 4). The general signs obtained using cell wall suggest the presence of an endotoxin (1, 4, 9, 16, 17).

The results in Experiment II support those obtained in cattle. The hemorrhagic conjunctivitis in mice and the periocular edema, myosis, ocular pruritus and lacrimation in cattle (Experiment Ic) that were given cell sap suggest the presence of a specific oculopathic substance associated with M. bovis. This is further substantiated by the induction of conjunctivitis in guinea pigs by cell sap. If an oculospecific substance is associated with *M. bovis* infection, this could present a mechanism through which keratoconjunctivitis is induced. However, that contention, along with the question of whether the oculopathic effect is related to the other toxic substances, remains to be determined. The findings in guinea pigs further enhance our belief that *M. bovis* produces at least two distinctly different toxins. This is intimated by the occurrence of composite signs in guinea pigs given equal part mixtures of cell wall and cell sap fractions, while those guinea pigs given only one fraction failed to develop all the signs. Also, under these circumstances, the guinea pigs given only mixed fractions would be getting only one-half the total dose of a specific toxic substance. Consequently, the effect of each should not be as severe. The expected results occurred, in that some guinea pigs died when inoculated with 0.5 ml cell sap, but none died when given 0.25 ml cell sap mixed with cell wall materials. Significantly, in another study[6] where density gradient isolated fractions of cell sap were used, the more purified material caused increased morbidity and mortality rates.

Possibly a third toxin could account for the anaphylactoid-like reactions in certain mice (Experiment IIa). This reaction appears to simulate the initial stages of strychnine poisoning in cats. More work is needed to explain this peculiar phenomenon.

Although the results obtained in this investigation could have been due to hypersensitivity reactions (4) in certain of the animals, this case is thought not to be true. This presumption is because animals (7) not previously exposed developed the same signs as animals previously exposed to *M. bovis*.

From the results of our study, it is concluded that *M. bovis* produces or is associated with two or more toxins which can cause deleterious effects when injected into cattle, mice and guinea pigs. This reaction makes cautious use of vaccine against BIK of paramount importance. Possibly these toxins can be neutralized by use of certain chemical or physical influences (10, 11) to facilitate their use in vaccines. Special precautions also should be taken to prevent the demonecrotoxin of *M. bovis* from producing lesions at the site of injection (7, 12) which could lead to subsequent condemnation at slaughter.

Although this study introduces evidence that *M. bovis* produces endotoxins as well as exotoxins, more definitive work is needed to substantiate these results. Also, findings of this study leave to speculation whether the pathological manifestations in BIK are related to a toxin or toxins produced by *M. bovis*.

ACKNOWLEDGMENTS

The assistance of Dr. Billy L. Deyoe in the interpretation of the pathophysiological findings of our results is gratefully acknowledged. Thanks are also expressed to Dr. Richard Merkal for his assistance in the preparation of fractions of *M. bovis*.

REFERENCES

1. **COOPER, K. E.** Some physiological and clinical aspects of pyrogens. **In** Pyrogens and Fever — A Ciba Foundation Symposium. Wolstenholme and Birch, eds. pp. 5-21. Edinburgh and London: Churchill Livingstone. 1971.

[6]**Pugh, G. W., Jr., and D. E. Hughes, National Animal Disease Laboratory, North Central Region, Agricultural Research Service, U.S. Department of Agriculture, Ames, Iowa 50010 (Unpublished data, 1971).**

2. **ELLINGHAUSEN, H. C., JR.** Some observations on cultural and biochemical characteristics of **Leptospira pomona.** J. infect. Dis. **106:** 237-244. 1960.
3. **FREER, J. H. and M. R. J. SALTON.** The anatomy and chemistry of gram-negative cell envelopes. **In** Microbial Toxins. Vol. IV. Bacterial Endotoxins. G. Weinbaum, S. Kadis and S. J. Ajl, eds. pp. 67-126. New York and London: Academic Press. 1971.
4. **GRAY, DAVID F.** Immunology. New York: American Elsevier Publishing Company. 1970.
5. **HENSON, J. B. and L. C. GRUMBLES.** Infectious bovine kerato-conjunctivitis. II. Susceptibility of laboratory animals to **Moraxella (Haemophilus) bovis** cultures. Cornell Vet. **50:** 445-458. 1960.
6. **HENSON, J. B. and L. C. GRUMBLES.** Infectious bovine kerato-conjunctivitis. III. Demonstration of toxins in **Moraxella (Haemophilus) bovis** cultures. Cornell Vet. **51:** 267-284. 1961.
7. **HUGHES, D. E. and G. W. PUGH, JR.** Experimental bovine infectious keratoconjunctivitis: Effectiveness of intramuscular vaccination with viable **Moraxella bovis** culture. Am. J. vet. Res. **32:** 879-886. 1971.
8. **JACKSON, F. C.** Infectious keratoconjunctivitis in cattle. Am. J. vet. Res. **14:** 19-25. 1953.
9. **MILNER, K. C., J. A. RUDBACH and E. RIBI.** General characteristic. **In** Microbial Toxins. Vol. IV. Bacterial Endotoxins. G. Weinbaum, S. Kadis and S.J. Ajl, eds. pp. 1-66. New York and London: Academic Press. 1971.
10. **NOWOTNY, A.** Chemical detoxifications of bacterial endotoxins. **In** Bacterial Endotoxins — Proceedings of a Symposium held at the Institute of Microbiology of Rutgers. M. Landy and W. Braun, eds. pp. 23-37. New Brunswick, New Jersey: Institute of Microbiology, Rutgers — The State University. 1964.
11. **NOWOTNY, A.** Chemical and biological heterogeneity of endotoxins. **In** Microbial Toxins. Vol. IV. Bacterial Endotoxins. G. Weinbaum, S. Kadis and S.J. Ajl, eds. pp. 309-329. New York and London: Academic Press. 1971.
12. **PUGH, G. W., JR.** Characterization of **Moraxella bovis** and its relationship to bovine infectious keratoconjunctivitis. Thesis, Iowa State University. 1969.
13. **PUGH, G. W., JR. and D. E. HUGHES.** Inhibition of auto-agglutination of **Moraxella bovis** by 10% magnesium chloride. Appl. Microbiol. **19:** 201-203. 1971.
14. **PUGH, G. W., JR and D. E. HUGHES.** Infectious bovine keratoconjunctivitis induced by different experimental methods. Cornell Vet. **61:** 23-45. 1971.
15. **SETH, R. N. and P. CHANDRASEKARIAH.** Studies on infectious bovine kerato-conjunctivitis. Indian vet. J. **34:** 248-252. 1957.
16. **WARDLAW, A. C., L. BOORMAN and R. REID.** Assay of endotoxin by he hypothermic response of mice. Br. J. exp. Path. **52:** 198-208. 1971.
17. **WORK, E.** Production, chemistry and properties of bacterial pyrogens and endotoxins. **In** Pyrogens and Fever — A Ciba Foundation Symposium. Wolstenholme and Birch, eds. pp. 23-47. Edinburgh and London: Churchill Livingstone. 1971.

Book Review

BIOLOGICAL ACTIVITIES OF COMPLEMENT. *5th International Symposium of the Canadian Society of Immunology, Guelph, Ontario, August 1970. Edited by D. G. Ingram. (Guelph, Ont.). Published by S. Karger AG, CH-4000 Basel 11 (Switzerland). 1972. 288 pages. Price $23.55.*

In the preface, the editor mentioned: "This Symposium was designed to reflect present trends in complement research in the center of which is the biological and medical significance of the complement system". To achieve this, internationally known specialists reviewed the current status of research in their specialties and presented recent progress.

This book of 288 pages includes 22 papers, 64 figures, 39 tables and 770 references. It is divided into 5 sections as follows: I. The complement system; II. Cell membrane damage due to complement activity; III. Biologically active fragments of complement components; IV. Complement-induced changes in membrane surface properties; and V. Biology of complement components.

Complement is now being recognized as an important contributor to a number of biological phenomena including chemotaxis, phagocytosis, anaphylatoxin generation, adherence reactions of blood cells and tissue damage. The report of this symposium provides current knowledge of the biochemistry and of the biological consequences of the activity of this complex system of the blood.

This book contains a vast amount of useful condensed information. It should serve as a good reference for those engaged in research in immunology and related fields, as well as for teaching.

— *P. Boulanger.*